GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT INITIATION

Date: August 29, 1978

Project Title: Laser-Excited Raman Spectroscopy of Biopolymers

Project No: G-33-G03

Project Director: Dr. Nai-Teng

Sponsor: DHEW/PHS/NIH - National Institute of General Medical Sciences; Bethesda, MD 20014

Agreement Period: From 9/1/78 Until 8/31/79 (08 Year)

Type Agreement: Grant No. 2 R01 GM18894-08

Amount: $97,664 PHS Funds (G-33-G03)
5,140 GIT Contribution (G-33-332)
$102,804 Total

Reports Required: Annual Progress Reports with Continuation Applications; Terminal Progress Report upon Grant expiration

Sponsor Contact Person (s):

Technical Matters

John C. Dalton, Ph.D. (Dr. Marvin Cassman)
Deputy Assoc. Director for Program Activities
National Institute of General Medical Sciences
Bethesda, MD 20014

Phone: (301) 496-7463

Contractual Matters
(thru OCA)

Evelyn W. Carlin, Grants Mgt. Officer
Office of Associate Director Program Activities
National Institute of General Medical Sciences
Bethesda, MD 20014

NOTE: FOLLOW-ON TO PROJECT G-33-G02 (07 YEAR)

Defense Priority Rating: none

Assigned to: Chemistry (School/Laboratory)

COPIES TO:

- Project Director
- Division Chief (EES)
- School/Laboratory Director
- Dean/Director—EES
- Accounting Office
- Procurement Office
- Security Coordinator (OCA)
- Reports Coordinator (OCA)
- Library, Technical Reports Section
- EES Information Office
- EES Reports & Procedures
- Project File (OCA)
- Project Code (GTRI)
- Other _____

CA—3 (3/76)

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION

Date: September 6, 1979

Project Title: Laser-Excited Raman Spectroscopy of Biopolymers

Project No: G-33-G03

Project Director: Dr. Nai-Teng Yu

Sponsor: DHEW/PHS/NIH - National Institute of General Medical Sciences; Bethesda, MD 20014

Effective Termination Date: 8/31/79 (08 year)

Clearance of Accounting Charges: 8/31/79

Grant/Contract Closeout Actions Remaining:

_ Final Invoice and Closing Documents
_ Final Fiscal Report
_ Final Report of Inventions
_ Govt. Property Inventory & Related Certificate
_ Classified Material Certificate
x Other Annual Report of Expenditures (08 year) due no later than 11/30/79.

NOTE: FOLLOW-ON PROJECT IS G-33-G04 (09 YEAR).

Assigned to: Chemistry (School/Laboratory)

COPIES TO:

Project Director
Division Chief (EES)
School/Laboratory Director
Dean/Director—EES
Accounting Office
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Security Coordinator (OCA)
Reports Coordinator (OCA)
Research Property Coordinator (OCA)
Library, Technical Reports Section
EES Information Office
Project File (OCA)
Project Code (GTRI)
Other_____

CA—4 (1/79)

SECTION IV

G-33 GD3

APPLICANT: REPEAT GRANT NUMBER SHOWN ON PAGE 1 ⟶	GRANT NUMBER
SECTION IV—SUMMARY PROGRESS REPORT	GM 18894-09
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Last, First, Initial)	PERIOD COVERED BY THIS REPORT
Yu, -Nai-Teng	FROM: 09/01/78 — THROUGH: 06/30/79
NAME OF ORGANIZATION	
Georgia Institute of Technology	

TITLE (Repeat title shown in Item 1 on first page)
Laser-Excited Raman Spectroscopy of Biopolymers

1. List publications: (a) published and not previously reported; (b) in press. Provide five reprints if not previously submitted.
2. List all additions and deletions in professional personnel and any changes in effort.
3. Progress Report. (See Instructions)

1a. (i) R. H. Felton and Nai-Teng Yu (1978) "Resonance Raman Scattering from Metalloporphyrins and Hemoproteins" in The PORPHYRINS, Chapter 8 (pp. 347-393), Vol. III (D. Dolphin, Ed.) Academic Press, Inc.

(ii) R. Mathies and Nai-Teng Yu (1978) "Raman Spectroscopy with Intensified Vidicon Detectors: A Study of Intact Bovine Lens Proteins" J. Raman Spectros. 7, 349

(iii) J.F.R. Kuck, Jr. and Nai-Teng Yu (1978) "Raman and Fluorescence Emission of the Human Lens. A New Fluorophor" Exp. Eye Res. 27, 737.

(iv) L. D. Cheung, Nai-Teng Yu and R. H. Felton (1978) "Resonance Raman Spectra and Excited Profiles of Soret-Excited Metalloporphyrins" Chem. Phys. Letts. 55, 527.

(v) E. J. East, R.C.C. Chang, Nai-Teng Yu and J.F.R. Kuck, Jr. (1978) "Raman Spectroscopic Measurement of Total Sulfhydryl in Intact Lenses", J. Biol. Chem. 253, 1436

(vi) A. Lanir, Nai-Teng Yu and R. H. Felton (1979) "Conformational Transitions and Vibronic Couplings in Acid Ferricytochrome c. A Resonance Raman Study" Biochemistry, 18, 1656.

1b. (i) C. C. Askren, Nai-Teng Yu and J.F.R. Kuck, Jr. (1979) "Variation of the Concentration of Sulfhydryl along the Visual Axis of Aging Lenses by Laser Raman Optical Dissection Techniques" Exp. Eye Res. (in press).

(ii) Nai-Teng Yu, J.F.R. Kuck, Jr. and C. C. Askren (1979) "Red Fluorescence in Older and Brunescent Human Lenses" Invest. Ophthal. and Visual Sci. (in press).

(iii) A. Lanir and Nai-Teng Yu (1979) "A Raman Spectroscopic Study of the Interaction of Divalent Metal Ions with Adenine Moiety of Adenosine 5'-Triphosphate" J. Biol. Chem. (in press).

2. (i) Dr. A. Lanir, on leave from Tel-Aviv University, spent one year in this laboratory as a research scientist. The research scientist position is now replaced by Dr. R. B. Srivastava. Dr. Srivastava is a very excellent scientist with a diverse background training and experience. He received his Ph.D. in Physics from India and spent one year each at State University of New York at Albany (under Prof. Y. P. Myer) and Syracuse University (under Prof. G. H. Atkinson). He is a very valuable addition to this group. Recently he prepared various heme peptide model compounds and assisted in the assembly and testing of our new multichannel laser Raman system.

xc: Otis Rodgers

(ii) Dr. Charles Pace, a Ph.D. in Biochemistry from Emory University, has joined my group, working in the isolation, purification and resonance Raman studies of cytochrome b_{562}, a novel hemeprotein from E. coli with its x-ray structure determined recently (Mathews, Bethge, and Czerwinski, J. Biol. Chem. 254, 1699 (1979)). He plays a role which coordinates with Dr. Srivastava's studies on cytochrome hemepeptide model compounds.

(iii) Mr. M. Tsubaki, a M.S. from Osaka University, is employed as a research technician. He has the expertise in chemical modification and reconstitution of heme proteins, particularly hemoglobin and myoglobin systems. He is different from usual laboratory technicans in that he has his own ideas and is capable of carrying out experiments independently. He is a real asset in our studies of low frequency modes of hemeproteins using a multichannel laser Raman spectrometer.

(iv) Mr. C. C. Askren is working on his M.S. thesis in T7 DNA and RNA polymerase cross-linking experiments using UV pulsed lasers. His earlier project involved measurement of sulfhydryl variation along the visual axis in intact ocular lens. The technique is now called "laser Raman optical dissection technique".

2. Summary Progress Report:

(1) Objective

a. The overall objectives of the total project are: (a) to develop the techniques and procedures necessary to obtain and interpret the Raman spectra of biopolymers; (b) to derive significant structural information not obtainable by other research techniques; and (c) to correlate the structure-function relationship biomolecules.

b. Goals set for the current year: (a) to develop a multichannel laser Raman system suitable for the detection of weak low-frequency modes in heme proteins; (b) to prepare excitation profiles of low-frequency modes in hemoglobin and cytochrome c; (c) to set-up apparatus and techniques for preparing various authentic metalloporphyrins and modified hemeproteins suitable for resonance Raman investigations; and (d) to continue the development of techniques for lens proteins studies.

(2) Main Scientific Findings and Their Significance:

a. Resonance Raman spectra of nickel, chromium, and copper porphyrins, excited at their Soret maxima, show decreasing scattering intensity due to depolarized modes. This order correlates with diminished Q(0-0) absorption in the porphyrins. The data are interpreted in terms of Jahn-Teller distortions in both Q and B states. General agreement between predicted and observed excitation profiles of nickel etioporphyrin I support a vibronic treatment of the excited states. We first demonstrated the importance of 180° scattering with a cylindrical lens in the measurement of resonance Raman intensity, when pulsed lasers are employed.

b. In corroboration with Prof. Mathies, a cooled SIT (silicon intensified target) was incorporated in a novel mismatched subtractive dispersion double monochromator that has optimum stray light ($<5 \times 10^{-9}$), throughput, and resolution for the studies of ocular lens proteins. It is demonstrated that the SIT-OMA detection technique is capable of obtaining high-quality Raman spectra of an intact bovine lens with a laser power

(~1 mW) significantly below the threshold for retinal damage.

c. During the past year, we put our major efforts into the development of a multichannel Raman system well suited for the detection of weak Raman signals in hemeproteins. The Raman system consists of a cooled silicon intensified target (SIT) detector, a PAR model 1215 OMA II console, a PAR model 1216 multichannel detector controller, and a SPEX 1402 double monochromator (two 600 g/mm gratings in additive dispersion). The performance of this system has been very satisfactory. We are now able to produce high-quality low frequency Raman spectra of hemeproteins with low laser power (~20 mW) in the Q bands region. A typical spectrum (200-550 cm^{-1}) of ferrocytochrome c excited at 530.9 nm (integration time ~5 min.) is shown in Figure 1. This excellent new Raman system is expected to generate much interesting and exciting results from heme proteins.

d. The Fe-O_2 stretching vibration at 570 cm^{-1} in oxyhemoglobin was first detected in the Q-bands region where no charge-transfer transition has been suggested. We have completed the measurement of excitation profile of this 570 cm^{-1} line in the visible wavelength region (450-580 nm) and now being extended into the near UV region (350-450 nm). In view of recent studies by Eaton et. al. (JACS, 100, 4991, 1978)) and by Churg & Makinen (JCP, 68, 1913 (1978)), this vibration in hemoglobin and myoglobin is of considerable interest.

e. Laser Raman optical dissection technique was employed to measure the variation of sulfhydryl levels along the visual axis (VA) of the lenses of different ages. The VA length of each rat and mouse lens was measured using a translation stage micrometer and the laser scattering. The rat VA length varied from 2.85 mm (26 days) to 4.32 mm (16 months). Spectra were obtained for 20 increments along the VA. The salient features in the series of curves are maxima in the cortex (one anterior, one posterior) and a central minimum. The youngest rat lens (maintained at 37° to avoid cold cataracts) showed a bell-shaped curve. All curves were nearly symmetric for the rat, the minima slightly off center for the mouse. The two maxima of the second youngest lens were separated by a distance of 1.55 mm which increased to 2.95 mm in the oldest lens. In about 7½ month lens a 0.78 mm segment of the VA center contained too little sulfhydryl to be detected by this technique. This segment increased to 1.44 mm in the oldest lens. The apparent rate of decrease in SH, being quite pronounded in the nucleus, is different at other points along the VA. A plot of sulfhydryl level vs. age for several points at distance r from the center (VA midpoint) along the VA indicates a steady decrease in SH levels with age for $r < 1.2$ mm. For larger r, there is actually an increase in SH. These results are interpreted in terms of 2SH → S-S conversion, changing rates of synthesis of the different crystallins and of glutathione synthesis along the VA.

3. Research Goals in the Coming Year:

a. To complete the measurement of excitation profile of the 570 cm^{-1} of Fe-O_2 stretching vibration in oxyhemoglobin.

b. Theoretical analysis of the Fe-O_2 excitation profile.

c. To compare low-frequency resonance Raman spectra of cytochrome b_{562} and cytochrome c making possible assignments to Fe-axial ligand modes to cytochrome systems, particularly the Fe-S or Fe-N_ε stretching vibrations.

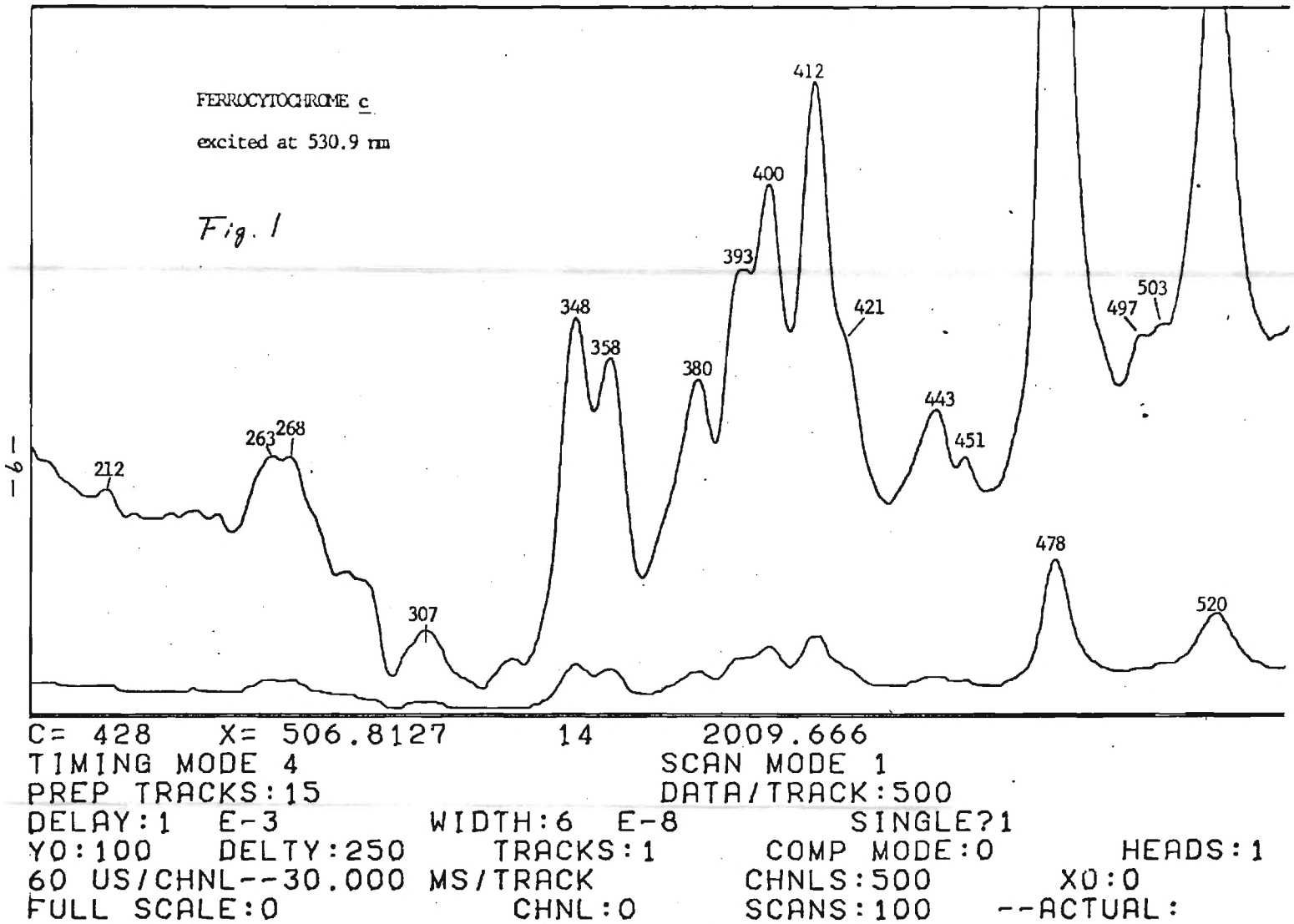
d. To develop effective cross-linking between T7 DNA and RNA polymerase, using ultraviolet pulsed lasers.

e. To initiate resonance Raman studies on cobalt- and manganese-substituted myoglobin and hemoglobin, particularly in the low frequency region.

The undersigned agrees to accept responsiblity for the scientific and technical conduct of the project and for provision of required progress reports if a grant is awarded as the result of this application.

6/13/74
DATE

Mei-Feng Yu
Principal Investigator


FERROCYTOCHROME c
excited at 530.9 nm
Fig. 1
-9-
212
263
268
307
348
358
380
393
400
412
421
443
451
478
497
503
520
C= 428 X= 506.8127 14 2009.666
TIMING MODE 4 SCAN MODE 1
PREP TRACKS:15 DATA/TRACK:500
DELAY:1 E-3 WIDTH:6 E-8 SINGLE?1
Y0:100 DELTY:250 TRACKS:1 COMP MODE:0 HEADS:1
60 US/CHNL--30.000 MS/TRACK CHNLS:500 X0:0
FULL SCALE:0 CHNL:0 SCANS:100 --ACTUAL:

Department of Health, Education, and Welfare

NAME AND ADDRESS OF GRANTEE INSTITUTION	TRANSACTION NO.	Grant No.
orgia Institute of Technology lanta, Georgia 30332	(08)R1GM18894C INSTITUTIONAL ID NO. G-33-G03	2 R01 GM18894-08 DATE OF THIS REPORTING PERIOD FROM 9/1/78 TO 8/31/79 PROJECT PERIOD FROM 9/1/78 TO 8/31/82 ☐ CHECK IF FINAL REPORT

Expenditures of DHEW Funds for this Reporting Period

. Personnel	$ 22,349.61	h. Alterations and renovations	
. Consultant services	730.00	i. Other	
. Equipment	39,344.92		
. Supplies	8,096.69	j. Total direct costs	71,164.07
. Travel, domestic	642.85	k. Indirect costs: Rate 76 % ☒ S&W ☐ TDC Base $ 22,349.61	16,985.70
. Travel, foreign			
. Patient care costs		l. TOTAL	$ 88,149.77

Expenditures from Prior Periods (previously reported)	128,638.74
Cumulative Expenditures	216,788.51
Total Amount Awarded — Cumulatively	236,340.00
Unexpended Balance (Item 4 less Item 3)	19,551.49
Unliquidated Obligations	
Unobligated Balance (Item 5 less Item 6)	
Cost Sharing Information — Grantee Contribution This Period	5,140.00
% of Total Project Costs (Item 8a divided by total of Items 1 and 8a)	% 6.0
Interest/Income (enclose check)	
Other Refundable Income (enclose check)	

Remarks

ereby certify that this report is true and correct to the best of my knowledge, and that all expenditures reported herein have been made in ordance with appropriate grant policies and for the purposes set forth in the application and award documents.

Dr. N. T. Yu — Nai-Teng Yu, Associate Professor — Jan. 23, 1980
Date

David V. Welch — 1/29/80
SIGNATURE OF INSTITUTION OFFICER — DATE

David V. Welch, Manager, Grants & Cont. Accounting

W-489 (REV. 10/73) — REPORT OF RESEARCH GRANT EXPENDITURES — OMB 85R0219

404-894-4624